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Video Article

Insertion of Flexible Neural Probes Using Rigid Stiffeners Attached with Biodissolvable Adhesive

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Abstract

Microelectrode arrays for neural interface devices that are made of biocompatible thin-film polymer are expected to have extended functional lifetime because the flexible material may minimize adverse tissue response caused by micromotion. However, their flexibility prevents them from being accurately inserted into neural tissue. This article demonstrates a method to temporarily attach a flexible microelectrode probe to a rigid stiffener using biodissolvable polyethylene glycol (PEG) to facilitate precise, surgical insertion of the probe. A unique stiffener design allows for uniform distribution of the PEG adhesive along the length of the probe. Flip-chip bonding, a common tool used in microelectronics packaging, enables accurate and repeatable alignment and attachment of the probe to the stiffener. The probe and stiffener are surgically implanted together, then the PEG is allowed to dissolve so that the stiffener can be extracted leaving the probe in place. Finally, an *in vitro* test method is used to evaluate stiffener extraction in an agarose gel model of brain tissue. This approach to implantation has proven particularly advantageous for longer flexible probes (>3 mm). It also provides a feasible method to implant dual-sided flexible probes. To date, the technique has been used to obtain various *in vivo* recording data from the rat cortex.

Video Link

The video component of this article can be found at http://www.jove.com/video/50609/

Introduction

Microelectrode arrays are an essential tool in neuroscience as well as emerging clinical applications such as prosthetics. In particular, penetrating micro-electrode probes enable stimulation and recording of neuronal activity through close contact with cells in the brain, spinal cord, and peripheral nerves. A major challenge for implanted neural probes is stability and longevity of the stimulation and recording functions. Modeling and experimental studies of the interaction between microelectrode probes and neural tissue have suggested that one mechanism for degradation is micro-tearing of neural tissue due to slight relative motion between the probe and tissue [1-3]. One solution is to fabricate flexible probes that match more closely the bulk stiffness properties of neural tissue in order to minimize relative micromotion. As such, biocompatible thin film polymers such as polyimide and parylene have been adopted as favorable substrates for microelectrode probes [4-8].

A tradeoff of flexible probes is that they are difficult to insert into the neural tissue. Researchers have taken various approaches to facilitate insertion of flexible probes while preserving the desirable mechanical properties. One class of designs modifies the polymer probe geometry to increase stiffness in certain sections or axes while maintaining compliance in other parts. This has been accomplished by incorporating ribs or layers of other materials [9,10]. Another approach integrates a 3-D channel into the polymer probe design that is filled with biodegradable material [11]. This probe can be temporarily stiffened, and after insertion the material in the channel dissolves and drains out. However, methods such as these that permanently modify the geometry of the final implanted device may compromise some of the desirable features of the flexible probe.

One method that does not alter the final probe geometry is to encapsulate the polymer device with biodegradable material to temporarily stiffen the device [12-14]. However, typical biodegradable materials have Young's moduli orders of magnitude smaller than that of silicon and would consequently require larger thickness to achieve the same stiffness. Adequately coating the probe can result in a more rounded or blunt tip, making insertion more difficult. Also, since dissolvable coatings are exposed, there is a risk of them dissolving immediately upon contact, or even close proximity, with the tissue.

Another class of methods uses novel probe substrate materials that reduce in stiffness after being implanted into tissue. Such materials include shape memory polymers [15] and a mechanically adaptive nanocomposite [16]. These materials are able to decrease in elastic modulus significantly after insertion, and can result in probes that more closely match the mechanical properties of neural tissue. However, the achievable range of

stiffness is still limited, so they may not be able to provide very high stiffness equivalent to silicon or tungsten wires. Thus in the case of flexible probes that are very long (*e.g.* >3 mm) or that have extremely low stiffness, a method of temporarily attaching a more rigid stiffener may still be required.

Yet another promising method reported is to coat a stiffening shuttle with a permanent self-assembling monolayer (SAM) to customize the surface interaction between the shuttle and the flexible probe [17]. When dry, the probe adheres to the coated shuttle electrostatically. After insertion, water migrates onto the hydrophilic surface, separating the probe from the shuttle so that the shuttle can be extracted. Shuttle extraction with reduced probe displacement was demonstrated (85 µm). However, with only electrostatic interactions holding the probe to the shuttle, there is some risk of probe slippage relative to the shuttle before and during insertion.

We have developed a method in which the flexible probe is attached to a stiffener with a temporary biodissolvable adhesive material that securely holds the probe during insertion. The probes used were made of polyimide, which has an elastic modulus on the order of 2-4 GPa. The stiffener was fabricated from silicon, with an elastic modulus of ~200 GPa. When attached, the stiffness of the silicon dominates, facilitating insertion. Once inserted into the tissue, the adhesive material dissolves and the stiffener is extracted to restore the probe to its initial flexibility. We selected polyethylene glycol (PEG) as the biodissolvable adhesive material. PEG has been used in implanted applications such as neural probes, tissue engineering, and drug delivery [11,18,19]. Some evidence has suggested that PEG may attenuate neuroinflammatory response in brain tissue [18,20]. Compared to other possible materials, including sucrose, poly lactic-co-glycolic acid (PLGA), and polyvinyl alcohol (PVA), PEG has a dissolution time in biological fluids that is of an appropriate scale for many implant surgeries (on the order of tens of minutes, depending on molecular weight). In addition, it is solid at room temperature and liquid at temperatures ranging from 50-65 °C. This property makes it particularly suitable for our precision assembly process. Moreover, similar to the SAM described in [17], the dissolved PEG is hydrophilic, facilitating extraction of the stiffener. This advantageous approach is enabled by a novel stiffener design and methodical assembly process which ensure uniform adhesive coverage and accurate and repeatable alignment. In addition to the assembly process, we present the method of implementing the removable stiffener during surgery, as well as an *in vitro* procedure to evaluate extraction of the stiffener.

The protocol presented herein assumes that the user possesses a flexible polymer microelectrode probe. The part of the protocol relating the fabrication of the stiffener and assembly of this probe to a stiffener assumes access to common tools found in a microfabrication facility. The protocol relating to insertion and extraction would likely be performed in a neuroscience-oriented laboratory.

Protocol

1. Assembly of Probe to Stiffener

This section of the protocol describes fabrication of a silicon stiffener, and the assembly of a thin-film polymer probe to the stiffener. **Figure 1** illustrates a typical polymer neural probe along with the proposed stiffener. The details of the stiffener design are shown in **Figure 2**. The novel feature of this design is the shallow "wicking" channel running along its length which is used to distribute liquid adhesive during assembly. The wider portion of the stiffener is a tab for handling during assembly and surgical insertion. A reservoir on the tab connects to the channel. The component is fabricated from silicon using standard microfabrication processes.

1. The silicon stiffener with a wicking channel was fabricated from a silicon-on-insulator (SOI) wafer with a device layer thickness equal to the desired thickness of the stiffener (**Figure 3A**). A reasonable range of stiffener thickness is 20-100 µm. It is recommended that the width of the stiffener be 20-30 µm smaller than the probe width, which helps to prevent overflow of the adhesive from the bond interface to the top of the probe. First the wicking channels are dry-etched using the standard Bosch process (**Figure 3B**). Next, the stiffener geometry is defined by a longer etch that stops on the buried oxide layer (**Figure 3C**). Finally, the stiffeners are released by wet-etching the buried oxide layer in 49% hydrofluoric acid (**Figure 3D**). After thoroughly rinsing the stiffeners, soak them in deionized water for 15 min.
2. Place a pellet of polyethylene glycol (PEG) of molecular weight 10,000 g/mol into the reservoir (**Figure 4**). Heat the stiffener to 65 °C so that the PEG melts and wicks into the channel by capillary action. Then cool to room temperature to solidify.
3. **Figure 5** shows a schematic of the flip chip bonder set up. Place the stiffener upside down on the base stage of the flip chip bonder, then pick up the stiffener with the tool head. Place the probe upside down on the base stage. Using the flip chip bonder, align the stiffener and the probe and then lower the stiffener and place it onto the probe.
4. The base stage of the flip chip bonder should have a heating element to apply heat to the substrate. After placing the stiffener, heat the assembly once again to 65 °C. Allow one minute for the PEG to remelt and begin to fill in the interface between the probe and stiffener. Cool to solidify.
5. Turn the assembly over and inspect from the top. Reheat as needed to allow the PEG to completely fill the interface between the probe and the stiffener. This can be visually evaluated since the probe is transparent. As the assembly is sitting on the heater top- (probe-) side up, manually place 1-3 extra pellets of solid PEG onto the tab so that they melt over the probe, providing additional reinforcement in this region (**Figure 6**). Finally, allow the assembly to cool so that the PEG solidifies. At this point, the assembly is ready for surgical insertion.

2. Insertion and Extraction

1. Mount the probe-stiffener assembly to a micromanipulator as illustrated in **Figure 7A** by adhering the back of the stiffener to the micromanipulator arm at the tab region. This may be done with double-sided tape or cement, but take care not to contact the probe with adhesive. Temporarily secure the connector end of the probe to the micromanipulator with a small piece of adhesive putty such that it can be easily removed with low force.
2. Position the probe assembly over the target and insert the probe with the desired insertion speed. Insertion speeds of 0.13-0.5 mm/sec were used when developing this protocol.
3. Immediately remove the connector end of the probe from the micromanipulator gently and rest it on a nearby surface, such as a second manipulator arm (**Figure 7B**). This must be done before the PEG begins to dissolve to avoid displacing the probe.

4. Allow time for PEG to dissolve. This amount of time will depend on PEG molecular weight and area of contact between the probe and stiffener. For example, with PEG molecular weight of 10,000 g/mol, a microelectrode probe about 6 mm and a matching stiffener that is 306 µm wide, 15 min has been found to be an adequate amount of time. Section 3 of the protocol presents a method to test the required dissolution time. During this time, apply phosphate buffered saline (PBS) using a dropper around the tab and insertion point to dissolve any PEG that is above the target (**Figure 7C**).
5. Using a motorized micropositioner, begin extraction of the stiffener by applying a displacement of 100 µm at a speed of 5 mm/sec. This initial fast motion helps to overcome any static friction and minimize probe displacement. Then, complete the stiffener extraction at a slower speed of approximately 0.1 mm/second (**Figure 7D**).
6. In the case of an actual surgery, continue with normal procedures to apply gel, silicone, and/or dental acrylic at the insertion site to secure and protect the probe, as demonstrated in [21].

3. Agarose Gel Test

This section of the protocol describes a set up and procedure to examine the extraction of the stiffener in a 0.6% agarose gel that approximates the bulk mechanical properties, pH, and salinity of brain tissue [17,22]. Since the gel is nearly transparent through short distances, stiffener separation and probe displacement can be observed.

1. Prepare a solution of 0.6% agarose in phosphate buffered saline (PBS). Mix at an elevated temperature to completely dissolve the agarose powder. Pour the solution into a shallow acrylic box; gel should be 3/4- 1 in deep. Allow to the gel set at room temperature for an hour.
2. Ensure that the hardened gel is saturated with PBS so that it does not dry out, and heat the gel to 37 °C.
3. Set up the micromanipulator, box of agarose gel, and microscopic camera system as shown in **Figure 8**.
4. Insert a glass reference fiducial into the box of gel by sliding it between the gel and the side of the box (**Figure 8**). Use a dental pick to square the features on the reference fiducial to the field of view of the digital microscope.
5. Mount the probe assembly to the micromanipulator as described in step 2.1.
6. Position the probe assembly over the gel about 1 mm behind the reference fiducial.
7. Insert the probe into the gel, using the camera to guide it to a desired depth in the field of view.
8. Immediately move the connector end of the probe to rest on a nearby surface.
9. Make any required adjustments to the camera image to focus on the probe (the reference fiducial features may be slightly out of focus). Take a snapshot of the probe location.
10. Allow PEG to dissolve (this time may vary, and in fact may be a parameter to be tested). Apply PBS near the tab to dissolve PEG that is above the gel.
11. Start video capture if desired, and begin extraction of the stiffener as described in step 2.5. When extraction is complete, take a final snapshot of probe location.
12. Use image processing tools to compare the images before and after stiffener extraction. Use the features on the reference fiducial that are visible in the field of view to register (align) the images. Calibrate the scale of the image based on the size of known features on the probe. Measure the distance of probe displacement.

Representative Results

This insertion technique was used in conjunction with LLNL thin-film polyimide probes, which have passed ISO 10993 biocompatibility standards and are intended for chronic implantation. A typical thin-film polyimide probe is illustrated in **Figure 1** along with a silicon stiffener that is approximately 10 mm long in the narrow region. This stiffener has one wicking channel running along its length, as shown in **Figure 2**. **Figure 3** illustrates the mimcrofabrication process used to create this stiffener out of silicon. **Figure 4** shows a pellet of solid PEG that was placed into the reservoir of the tab, as seen through the camera on the flip chip bonder system. Once it was heated using the heater built into the base stage of the flip chip bonder, the PEG melted and began to wick into the channel. The camera view allowed us to monitor the wicking process until the PEG completely filled the channel, which took approximately an hour with PEG of molecular weight 10,000 g/mol. The PEG was then resolidified and the probe and stiffener were set up in the flip chip bonder as shown in **Figure 5**. **Figure 9A** shows a top view of a probe and stiffener after being aligned and attached, with PEG completely filling the interface. **Figure 9B** shows an example of an air bubble where PEG is not present because of a particle. The final step in the assembly is to add PEG to the tab region over the cable part of the probe, for extra reinforcement during handling. Since this area will not be inserted into the target, it is acceptable to have a larger volume of PEG here, as shown in **Figure 6**. This assembly method has been used to attach various shapes of probes to stiffeners, including multi-shank devices, as in shown in **Figure 10**.

The *in vitro* agarose gel test has been used to qualitatively evaluate different parameters such as PEG molecular weight, time allowed for PEG to dissolve, and stiffener geometry. With each combination of PEG and stiffener geometry, a set amount of time was allowed for dissolution. Then, extraction was attempted while observing probe displacement in real time. If the probe was dragged significantly (>200 µm) without visibly separating or sliding relative to the stiffener, we concluded that the PEG was not fully dissolved. **Table 1** gives some representative observations of PEG dissolution with varying times and varying molecular weight with a stiffener that is 6 mm long and 306 µm wide. Another observation in subsequent tests was that when the stiffener is more narrow (*e.g.* 220 µm), the PEG was dissolved in less time (as little as 5 min). This is likely because the adhesive contact area was decreased and as a result, there was a smaller volume of PEG to dissolve. Parameters that did not appear to affect PEG dissolution or probe displacement were stiffener thickness (thickness ranging from 20 µm to 100 µm were tested) and number of wicking channels (1 vs. 3).

The *in vitro* test has also been used to quantify average probe displacement for a given probe/stiffener/adhesive configuration. In this example, the test was performed using the insertion/extraction sequence illustrated in **Figure 7** wherein the probe-stiffener assembly is inserted into the agarose gel, the connector end is moved to a nearby surface, the PEG is allowed to dissolve, and the stiffener is finally extracted leaving the probe in place. The experimental set up in **Figure 8** shows the probe-stiffener assembly attached to the micromanipulator arm and positioned

over the gel. The reference fiducial was a small glass chip with an array of gold dots placed against the acrylic box in the field of view of the digital microscope.

The devices in this example were approximately 10 µm thick and 536 µm wide with eight 100-µm diameter electrodes. The traces consisted of a Ti-Au-Ti metal stack. The silicon stiffener was 220 µm wide, 6 mm long and 50 µm thick. The probe-stiffener assembly was inserted approximately 5 mm into the gel. **Figure 11** shows snapshots before and after extraction of a stiffener from a probe assembly that was tested in agarose gel. The light gold features in the images are from the reference fiducial and were used as reference features to align the images to each other. The known pitch between the electrodes (200 µm) was used to calibrate the pixel size, since this dimension is less sensitive to variations in the fabrication process. The net probe displacement due to stiffener extraction was estimated to be 28±9 µm (mean ± standard error, *n*=5).

To date, the proposed method has been extended to actual animal surgery on several occasions to implant a probe into a rat cortex. After assembly, the probe and 50-µm-thick stiffener were sterilized together in EtOH at room temperature. The insertion and extraction were performed with a micromanipulator attached to a stereotaxic frame. The probe-stiffener assembly was inserted at 0.13 mm/sec approximately 4 mm into the cortex of a rat. After 15 min, the stiffener was extracted, leaving the probe in place. After recovery from surgery, neural recordings, as shown in **Figure 12**, were successfully obtained from the awake animal demonstrating the viability of this method in real surgeries [23]. This implantation technique has also been used to obtain *in vivo* recordings with dual-sided arrays that have electrodes on both the front and back sides, as shown in **Figure 13**.

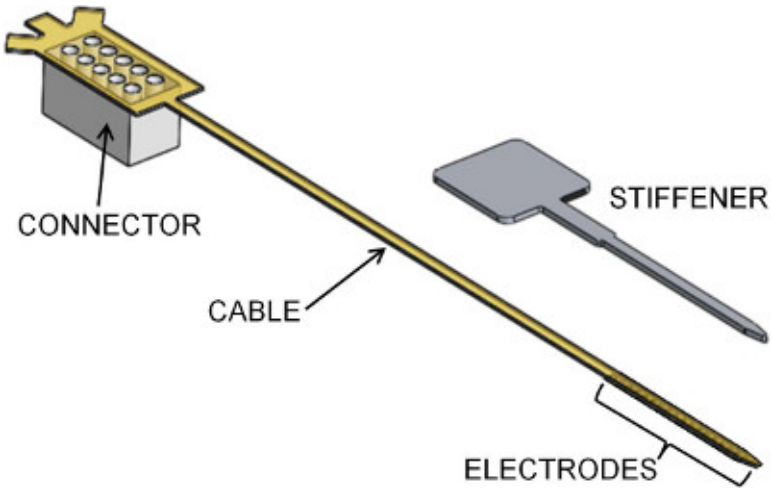

Figure 1. Schematic of a typical neural probe and the proposed stiffener. A typical thin-film polymer probe has one or more electrodes on the probe end. Metal traces run from the electrodes along the length of the cable portion and terminate on a pad that is attached to an electrical connector. The stiffener length (in this case approximately 10 mm) depends on the insertion depth of the probe, and a wider tab on the stiffener allows for handling. (*Image courtesy Diana George)*

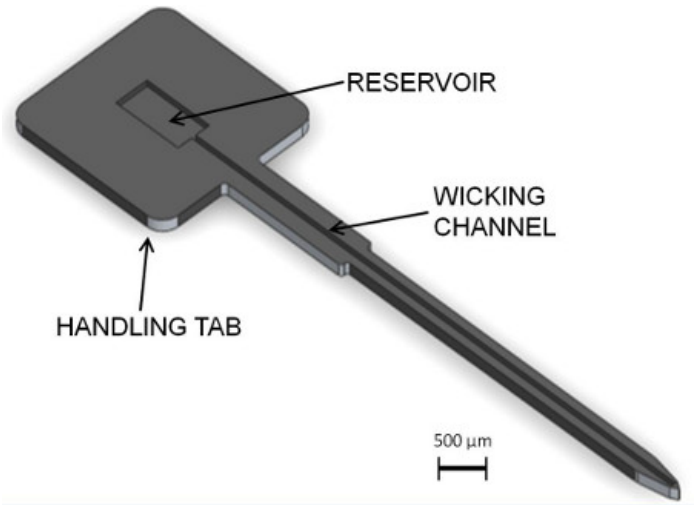

Figure 2. Stiffener design details. A wicking channel exploits capillary action to distribute a liquid adhesive that has been deposited into the reservoir. The reservoir is on a wider tab region that facilitates handling. (*Image courtesy Diana George)*

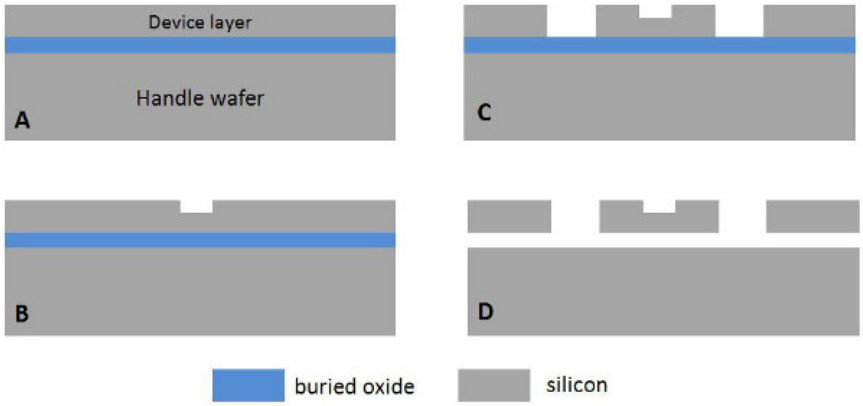

Figure 3. Fabrication sequence for silicon stiffener. The silicon stiffener is fabricated on a silicon-on-insulator (SOI) wafer **(A).** First the wicking channels are dry-etched using the standard Bosch process **(B).** Next, the stiffener geometry is defined by a longer etch that stops on the buried oxide layer **(C).** Finally, the stiffeners are released by wet-etching the buried oxide layer in 49% hydrofluoric acid **(D).**

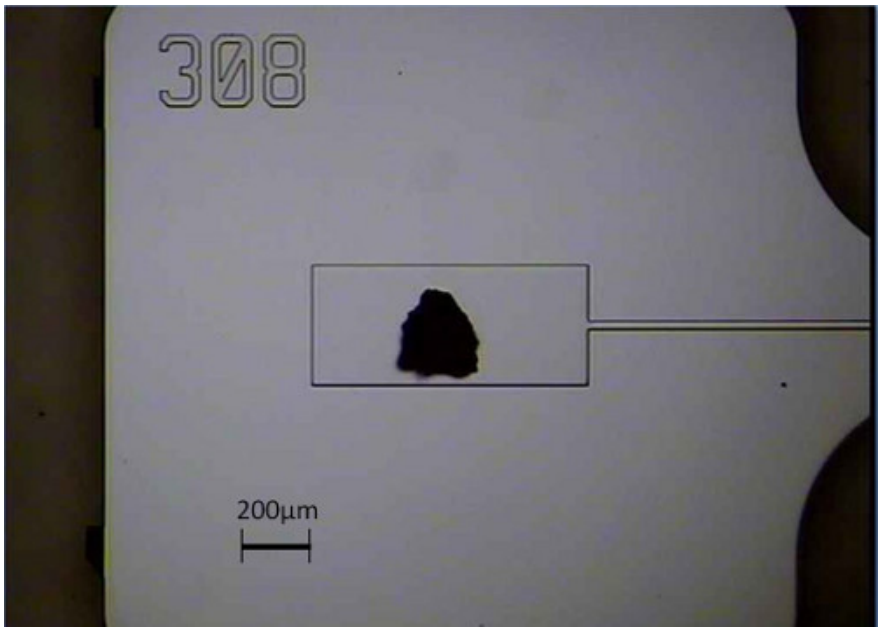

Figure 4. Polyethylene glycol in the stiffener reservoir. A flake of polyethylene glycol placed in the reservoir of the stiffener. Once heated, it will melt, fill the reservoir, and flow into the wicking channel.

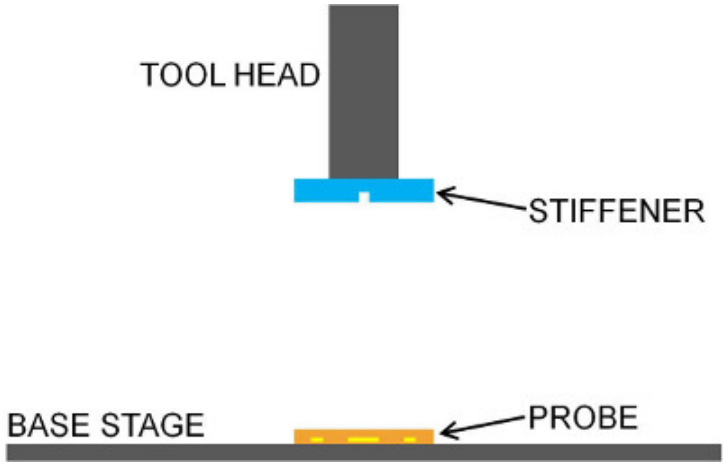

Figure 5. Schematic of flip-chip bonding. The stiffener is held with the channel down by a vacuum on the tool head of the flip-chip bonder. The neural probe lies on the base stage face down.

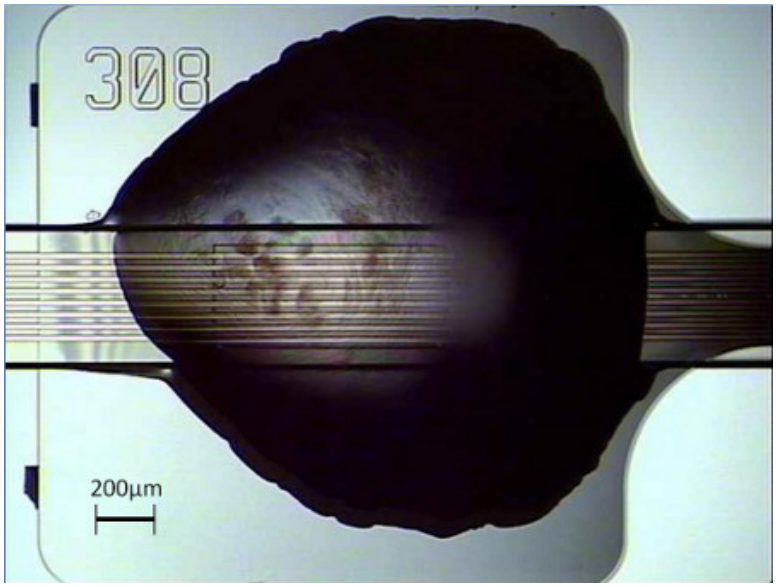

Figure 6. Polyethylene glycol on stiffener tab. Extra polyethylene glycol is generously applied on the tab of the stiffener as reinforcement. The cable portion of a polyimide probe is visible on top of the stiffener.

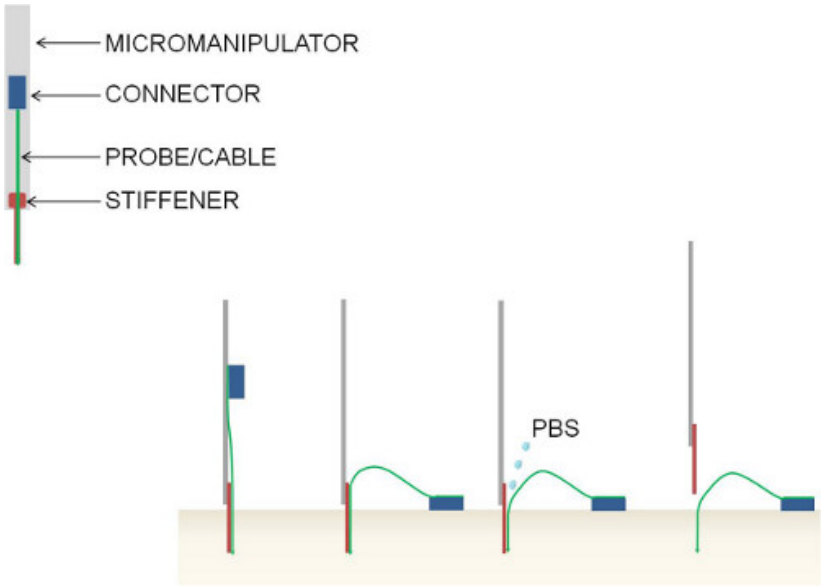

Figure 7. Schematic of insertion and extraction sequence. A) The probe-stiffener assembly is inserted into tissue using the micromanipulator. **B)** The connector end is moved to a nearby surface. **C)** PBS is apply to dissolve PEG on the tab of the stiffener. **D)** The stiffener is extracted, leaving the probe in the target.

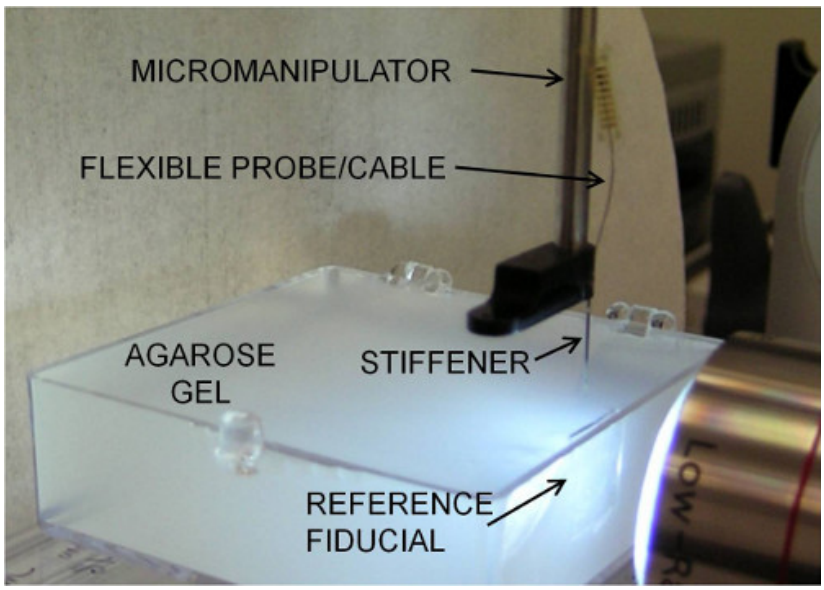

Figure 8. *In vitro* test set up. The set up for testing probe insertion and stiffener extraction in 0.6% agarose gel in phosphate buffered saline. The probe-stiffener assembly is attached to the micromanipulator arm and positioned over the gel target near the reference fiducial. A digital microscope is used to observe the probe and stiffener in the agarose gel.

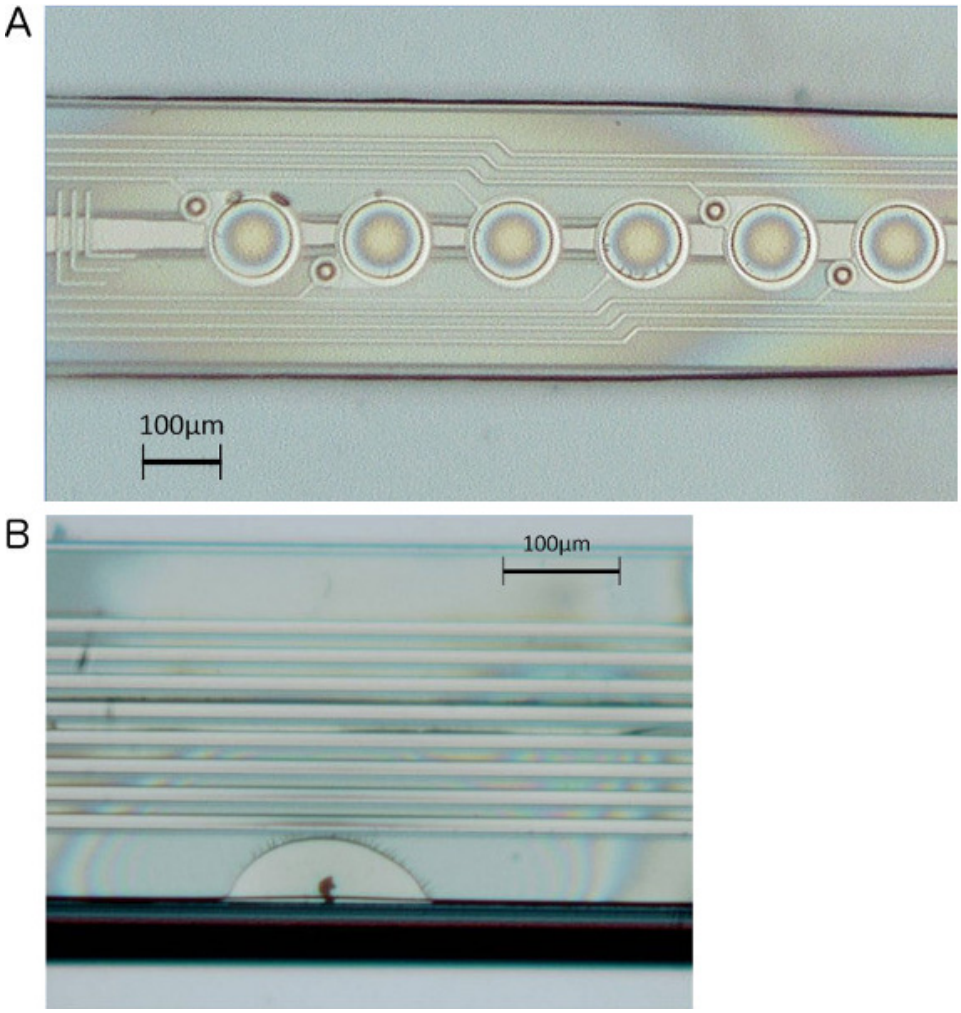

Figure 9. Probe adhered to stiffener. A) Top view of a probe attached to a stiffener with good alignment and complete adhesive coverage. **B)** An example of a gap in the adhesive coverage due to a particle.

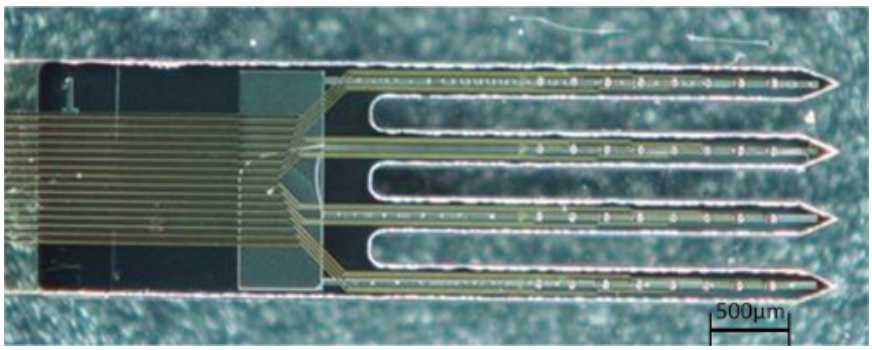

Figure 10. Example of a multi-shank probe. The proposed assembly process was used to attach this four-shank probe to a matching silicon stiffener.

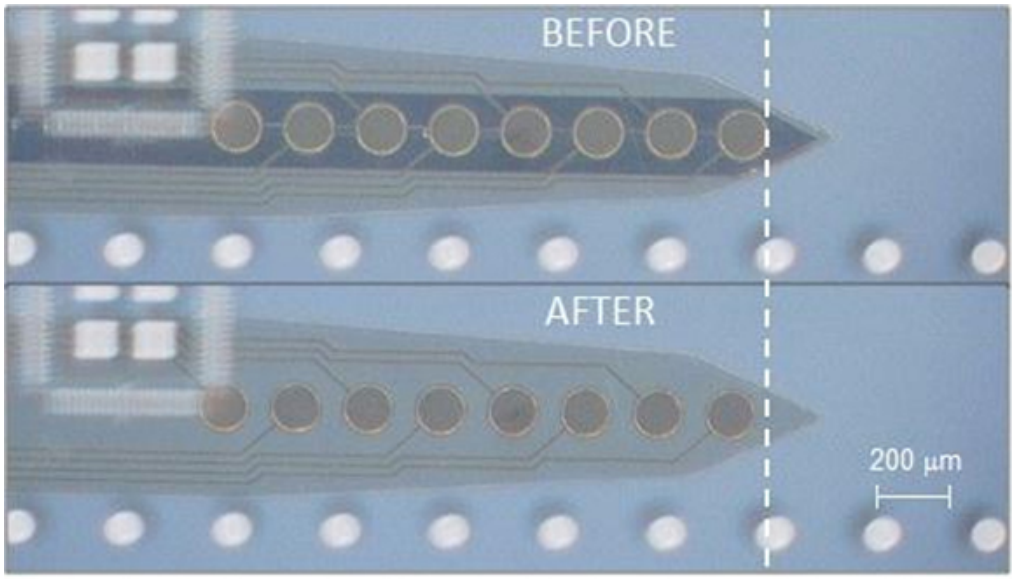

Figure 11. Example of stiffener extraction results. Snapshots from before (top) and after (bottom) stiffener extraction with a thin-film polyimide probe in agarose gel. The light gold dots are on the reference fiducial and are used as reference features to compare the images and measure probe displacement. The estimated displacement of the probe is 28±9 µm (mean ± standard error, *n*=5).

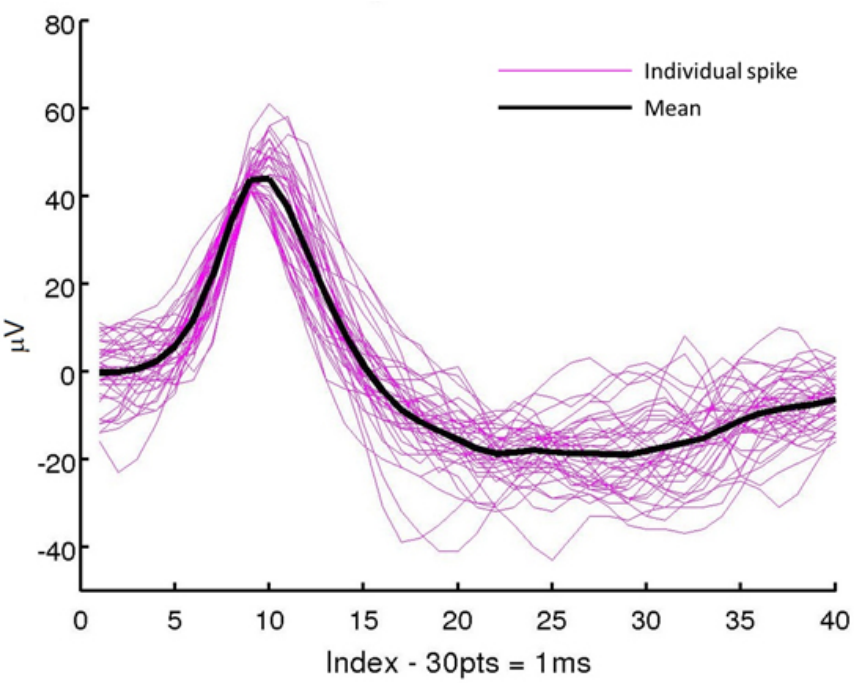

Figure 12. Example of physiological recordings. These single neuron spikes were obtained from a flexible microelectrode probe implanted with a removable stiffener as described in this protocol.

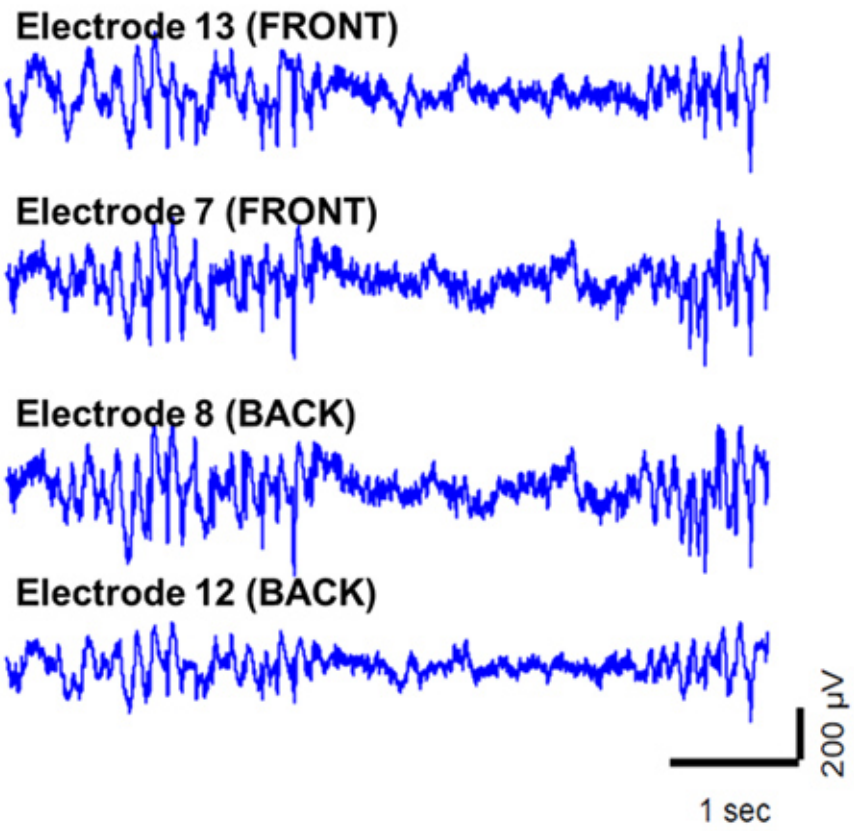

Figure 13. LFP recordings from a dual-sided probe. Insertion with a removable stiffener enabled testing of a flexible array that had electrodes on both the front and back surfaces. These LFP recordings demonstrated comparable electrode performance on both sides after implantation.

			PEG dissolved after:		
Probe length (mm)	**Stiffener width (µm)**	**PEG molecular weight (g/mol)**	**10 min**	**15 min**	**30 min**
6	306	6,000	yes		yes
		10,000		yes	
		20,000	no		yes

Table 1. PEG dissolution time in 0.6% agarose gel. Observations on dissolution of PEG of different molecular weights used to attach a flexible probe to a silicon stiffener, after varying amounts of time.

Discussion

The method described here provides a well-controlled process to attach thin-film polymer probes to separate stiffeners with a biodissolvable adhesive. Also presented is the recommended surgical procedure to implement these removable stiffeners and a technique to validate the procedure *in vitro* for a given probe-stiffener configuration. Since the stiffener can be made arbitrarily rigid, the method can facilitate insertion of relatively long probes (>3 mm). As such, the insertion method is expected to be an enabling technology for applications in deep brain stimulation (DBS), spinal cord stimulation, and peripheral nerve interfaces.

The novel stiffener with a wicking channel and the flip-chip based assembly process are suitable for various materials and probe configurations. Geometrically, the stiffener does not have to match the probe footprint and could, for example, be narrower than the probe. The thickness of the stiffener may also vary. While we have described a stiffener made from silicon, with other material, it may be possible to achieve more desirable mechanical properties for certain applications. The assembly process is also suitable for other types of liquid adhesive. PEG is particularly easy to work with because of its ability to be solidified and remelted multiple times. In the case of other liquid adhesives that do not have this property, the assembly sequence may need to be modified. It is possible to use a different molecular weight for the PEG. A higher molecular weight will take longer to dissolve, which may be desirable during surgery. The contact area between the probe and stiffener will also affect the time needed to dissolve the adhesive after probe insertion. It is recommended that the probe-stiffener configuration with the chosen molecular weight be tested *in vitro* as described in Section 3 to characterize the time required to dissolve the adhesive.

We found that precisely controlling the extraction speed is critical for extracting the stiffener with minimal probe displacement. Specifically, an initial quick motion helps to overcome static friction and separate the stiffener from the probe. After this, the remainder of the extraction can be

completed at a slower speed with negligible additional probe displacement, as observed in the agarose gel test. Many neuroscience laboratories use KOPF stereotaxic systems, and there is a motorized mircopositioner module from KOPF (*e.g.* Model 2662) that can be added to these systems. We chose a Newport motorized actuator because it had similar dynamic performance, but was less expensive and had more flexible speed control. (It was necessary to fabricate a simple bracket to attach the actuator to our micropositioner system.) The KOPF system can apply two extraction speeds similar to the protocol we developed. However, the maximum speed of the KOPF actuator is 4 mm/sec, while we used 5 mm/sec for the initial displacement using the Newport actuator.

During the *in vitro* and *in vivo* test, the insertion of the probe-stiffener assembly was performed either with a manually driven micromanipulator, or a motorized micromanipulator, with speeds ranging from 0.13-0.5 mm/sec. No damage to or delamination of the probe was observed. Higher insertion speeds have not been evaluated to determine risk of damage to the probe-stiffener assembly.

Modifications to the insertion/extraction procedure are in progress to make the process more robust. In particular, a very sensitive step is moving the connector end of the probe off of the micromanipulator onto a nearby surface. There is a risk in this step of disturbing the probe before it has been secured. It is also possible that the bend in the cable can cause stress on the inserted portion of the probe, leading to unintended displacement of the probe after stiffener extraction. Currently, these risks are mitigated by using a probe with a cable that is at least 2.5 cm long. However, it is desired that the insertion/extraction process be less dependent on the probe design. Modifications to the micromanipulator tool end or the addition of staging fixtures that can temporarily support the connector will likely allow more reliable extraction of the stiffener.

There are several open questions that could lead to future studies extending from this method. First, while the 0.6% agarose gel provided the best known *in vitro* brain tissue surrogate and allowed imaging analysis of probe displacement, it does not exactly replicate brain tissue. Studies are needed to examine location and displacement of the probe *in vivo*. Secondly, long term implantation and histological testing is needed to quantify benefits of the flexible probe with a removable stiffener. Such studies could investigate the theory that probe compliance reduces micromotion and extends electrode performance. Finally, it would be beneficial to more accurately characterize degradation rate of the PEG. This could assist in better tuning of dissolution times for particular surgical needs. Such measurements could also quantify how long the dissolved PEG remains between the probe and stiffener, which is important since the hydrophilic nature of the PEG facilitates extraction of the stiffener.

Disclosures

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